B8 allowing the cell introduced into the female animal to develop into a transgenic animal comprising a minichromosome [and

exposing the animal cell containing the λ neo-chromosome to conditions whereby a transgenic animal develops therefrom].

REMARKS

Any fees that may be due in connection with filing this paper or with this application may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 50-1213.

A unexecuted DECLARATION under 37 C.F.R. §1.132 of Perez is attached hereto. The executed original DECLARATION will be submitted upon receipt.

Claims 32-41, 43, 44, 59, 60, 65, 67, 71-74 and 82-105 are presently pending in this application. Claims 42, 64, 66, 68-70 and 75-81 have been cancelled without prejudice and will be pursued in a continuing application. Claims 32-34, 36-39, 41, 43, 44, 65, 67, 73 and 74 have been amended and claims 82-105 have been added in order to more particularly point out and distinctly claim the subject matter that applicant regards as the invention. No amendments have been made to obviate prior art and no new matter has been introduced. The amendments to claims 32-34, 36-39, 41, 43, 44, 65, 67, 73 and 74 find basis in the specification and claims as originally filed. Therefore, since the amendments change the form, not the substance of the claimed subject matter, no new matter has been added. Accordingly, entry of the amendments to the claims is respectfully requested.

Claims 82-105 have been added. These claims find basis in the specification as originally filed. Because the new claims find basis in the specification and the claims as filed, no new matter has been added. Accordingly, entry of the new claims in the case is respectfully requested.

THE REJECTION OF CLAIMS 32-41, 43, 44, 59, 60, 64-67 and 71-74 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 32-41, 43, 44, 59, 60, 64-67 and 71-74 are directed to methods of producing a transgenic animal. It is acknowledged in the Office Action that the previous Perez Declaration under 37 C.F.R. §1.132 filed March 31, 2000, in response to the previous Office Action (Paper No. 9 mailed August 17, 1999) shows the production of transgenic mice comprising a satellite DNA-based artificial chromosome that comprises heterologous DNA and thus is persuasive in demonstrating that the specification enables methods of using satellite DNA-based artificial chromosomes to produce transgenic mice expressing heterologous genes at detectable levels in so far as the method includes steps fully describing generation of the mice. Applicant notes that the data presented in the previous Perez Declaration (submitted on March 31, 2000), with respect to analysis of the transgenic mice, were from nucleic acid amplification analyses of tail DNA and FISH analysis of mitogen-activated peripheral blood lymphocytes, which confirmed the presence of the artificial chromosomes in cells of the mice. The data presented in the previous Perez Declaration showing positive results of X-gal staining, and thereby indicating the presence of a functional marker gene within the artificial chromosome, were from analyses of **transgenic preimplantation embryos.**

Although the previous Perez Declaration is acknowledged as persuasive, each of claims 32-41, 43, 44, 59, 60, 64-67 and 71-74 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of making a transgenic mouse using satellite DNA-based artificial chromosomes comprising at least one heterologous gene such that the gene is expressed at detectable levels, and wherein the method comprises specific steps leading to the production of the transgenic mouse, allegedly does not reasonably provide enablement for the claimed methods of producing transgenic animals of any and all species, wherein the method recites only that the embryonic cell is

exposed to conditions to produce the transgenic animal, and wherein the expression of the heterologous gene leads to an immunoprotective effect. Thus, it is alleged that the specification does not enable a person skilled in the art to which it pertains to make and/or use the invention commensurate in scope with these claims.

Specifically, it is asserted that evidence providing a nexus between a transgenic mouse and other transgenic animals by use of satellite DNA-based artificial chromosomes is necessary to enable the breadth of the claimed methods and that the evidence of record fails to provide the nexus. In particular, it is alleged that (1) the specification refers only to transgenic methodology pertaining to the production of transgenic mice and that the prior Office Action (Paper No. 9) cited references which support that transgene behavior in mice cannot be extrapolated to other species of animal and (2) the only embryonic stem cells that have been shown to give rise to germline tissues and a whole transgenic animal are **mouse** embryonic stem cells. Reconsideration of these grounds for the rejection is respectfully requested based on the following remarks.

Relevant law

In order to satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be satisfied by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples or by broad terminology." In re Marzocci et al., 469 USPQ

367 (CCPA 1971)(emphasis added). Patents are written to enable those of skill in the art to practice the invention. A patent need not disclose what is well known in the art (W.L. Gore & Assoc. v. Gorlock, Inc., 721 F.2d 1540, 1556, 220 USPQ 303, 315).

The inquiry with respect to scope of enablement under 35 U.S.C. § 112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims (i.e. the "Forman factors"). Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

It would not require undue experimentation to practice the claimed methods and make and use the claimed transgenic animals.

As discussed below, the claims are commensurate in scope with the disclosure, which exemplifies particular embodiments within the scope of the claims and also teaches how one of skill in the art can obtain other embodiments within the scope of the claims. In particular, there is an enormous amount of guidance presented in the specification, there are numerous working examples, the level of skill in the art is high, and the state of the prior art at the time of filing of the application was such that a large amount information concerning recombinant DNA techniques and procedures for the manipulation of DNA was available. Therefore, it would not require undue experimentation for one of skill in the art to make and use the claimed subject matter.

Evaluation of the above Factors

1. The scope of the claims

Claims 32-41, 43, 44, 59, 60, 64-67 and 71-74 are directed to methods of producing transgenic animals by introducing an artificial chromosome, and in particular a satellite artificial chromosome or a minichromosome generated by a prescribed method into an animal cell which is used in the generation of a transgenic animal.

The specification describes in extensive detail the preparation, characterization and isolation of artificial chromosomes, such as satellite artificial chromosomes and minichromosomes, and provides numerous examples of particular embodiments thereof. The specification further describes methods of incorporating heterologous DNA into artificial chromosomes, expression of the heterologous DNA in cells containing the artificial chromosomes and the use of such artificial chromosomes in the preparation of transgenic animals.

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. In the above-captioned application, Applicant discloses to the public methods and compositions for the controlled introduction and stable extra-genomic maintenance of large heterologous DNA fragments in cells without disruption of the inherent genome and, likewise, without the otherwise uncontrollable influences that the genomic DNA may have on the expression of the heterologous DNA. The artificial chromosomes disclosed in the application can be manipulated and used to express heterologous genes in cells, as is taught and specifically exemplified in the specification. It is clear that

Applicant's discovery is of a pioneering nature, and, as such, is entitled to broad claim protection.

As taught in the above-captioned application, any methods known in the art pertaining to introduction of foreign genes carried in traditional, standard sources (such as genes harbored in expression vectors) into cells for any variety of purposes, *e.g.*, gene therapy, protein production and the generation of transgenic animals, may be applied in similar fashion to the introduction of artificial chromosomes, particularly satellite DNA-based artificial chromosomes and minichromosomes, into cells. The application describes and demonstrates that once the artificial chromosomes are generated and isolated and/or introduced into cells, then any known procedure that has previously been carried out with any heterologous gene from any source is applicable to utilization of artificial chromosomes carrying foreign genes of interest. The application is replete with descriptions of numerous uses of the artificial chromosomes. The descriptions of the many ways in which the artificial chromosomes may be used include references to reported procedures for introducing exogenous nucleic acids into cells.

It is therefore respectfully submitted that the claims directed to methods of producing transgenic animals using the artificial chromosomes are commensurate in scope with the discovery and its disclosure within the above-captioned application. It would be unfair and contrary to the Constitutional mandate set forth in Article, Section 8, to deprive Applicant of protection of the broad applications of the pioneering discovery disclosed and described in exhaustive detail in the subject application.

2. Level of skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application, authored and reviewed by those known in the art, further evidences the high degree of skill in this art.

3. State of the art

At the time of filing of the application to which the subject application claims the benefit of priority, a broad body of knowledge had amassed in the area of molecular biology including many technical procedures covering the manipulation of DNA and recombinant DNA techniques. Numerous such procedures are referenced in the instant application, as illustrated in the response (Paper No. 10) to the previous Office Action. For example, procedures for the introduction of nucleic acids into cells, particularly for the production of transgenic animals, are referred to in many instances throughout the application.

Particular exemplary methods of generating transgenic animals that may be used in association with the artificial chromosomes are also described and referenced in the application. For example, the specification refers to procedures in which exogenous genetic material is introduced into a pronucleus of a mammalian zygote by microinjection [see, *e.g.*, U.S. Patent Nos. 4,873,191 and 5,354,674; see, also, International PCT application No. WO95/14769, which is based on U.S. application Serial No. 08/159,084] and the resulting embryo or zygote is transplanted into a host female uterus and allowed to develop. Exemplary microinjection and implantation procedures referred to in the specification include procedures described in Hogan *et al.* [(1994) *Manipulating the Mouse Embryo, A :Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY]. The specification also describes procedures whereby an artificial chromosome-containing cell is fused with mouse embryonic stem cells with reference to, *e.g.*, U.S. Patent No. 5,453,357, Hogan *et al.* (*supra*) and "Guide to Techniques in Mouse Development" in *Methods in Enzymology Vol. 25* [Wassarman and De Pamphilis, eds. (1993), pages 803-932].

Procedures for use in generating transgenic animals of various species, including but certainly not limited to murine species, were well established and routinely and successfully practiced on the date to which the instant application

claims priority (i.e., April 10, 1996). Prior to April 1996, numerous patents were issued which describe and claim methods of making transgenic animals, such as mice, and various transgenic mice [see, *e.g.*, U.S. Patent Nos. 5,387,742 (claiming transgenic mice expressing β amyloid protein), 5,434,340 (claiming transgenic mice T-cell receptor β chain polypeptide variant) and 5,491,283 (claiming transgenic mice carrying a BCR/C-ABL transgene)]. Additionally, numerous reports had published describing the generation of transgenic animals, such as mice [see, *e.g.*, Hogan *et al.* (*supra*); Gordon *et al.* (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*:7380; Brinster *et al.* (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*:4438; Palmiter *et al.* (1982) *Nature 300*:611-615; Gordon *et al.* (1987) *Bio/Technology 5*:1183; Simons *et al.* (1987) *Nature 328*:530; and Houdebine (1994) *J. Biotechnol. 34*:269-287].

Many of the procedures initially described for use in the generation of transgenic mice have been applied to the development of other transgenic animals, including livestock such as cattle and pigs, and numerous reports of non-murine transgenic animals had published by April 1996. Published reports of the generation of transgenic cattle include: Krimpenfort *et al.* (1991) *Bio/Technology 9*:844-847; PCT Application Publication No. WO93/25567; Haskell and Bowen (1995) *Mol. Reprod. Dev. 40*:386-390; and Hill *et al.* (1992) *Theriogenology 37*:222. Published reports of the generation of transgenic swine include: Wall *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*:1696; and Velander *et al.* (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*:12003. Published reports of the generation of transgenic sheep and goats include: Simons *et al.* (1988) *Bio/Technology 6*:179; Wright *et al.* (1991) *Bio/Technology 9*:830; and Ebert *et al.* (1991) *Bio/Technology 9*:835. Published reports of transgenic rabbits include: Buhler *et al.* (1995) *Bio/Technology 8*:140; and Riego *et al.* (1993) *Theriogenology 39*:1173. Non-mammalian transgenic animals that have been reported include transgenic *Drosophila* [see, *e.g.*, Rancourt *et al.* (1990)

Bio/Technology 8:453-457] and silkworm [see, *e.g.*, Maeda (1985) *Nature 315*:592-594].

These references to numerous published protocols for DNA manipulation, recombinant DNA expression and transgenic animal production demonstrate the large volume of information regarding tested and reliable procedures available at the time of filing of the application to which the instant application claims priority and thus evidence the advanced state of the art at the relevant time.

4. The amount of direction and guidance presented, and teachings in the specification

The claims are directed to methods of producing transgenic animals in which artificial chromosomes, such as satellite DNA-based artificial chromosomes or minichromosomes, are introduced into animal cells that are then used in the generation of the transgenic animal. As discussed in detail in the response (Paper No. 10) to the previous Office Action (Paper No. 9), with specific references to the subject application, the specification enables one of ordinary skill in the art to, by following the methods set forth therein, generate artificial chromosomes, readily identify the resulting artificial chromosomes based on the detailed characterization provided in the specification, incorporate foreign DNA into an artificial chromosome, isolate and transfer artificial chromosomes for use in other cells and systems and utilize the artificial chromosomes in the generation of transgenic animals. By virtue of Applicant's discovery of artificial chromosomes and the teachings of the specification, those of ordinary skill in the art are able, without undue experimentation, to make and use the artificial chromosomes and to combine the artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the generation of transgenic animals.

5. Nature of the invention

Clearly, Applicant's discovery of a means of producing synthetic chromosomes, the basic functional units common to all eukaryotes for the storage and transmission of vital genetic information, which are maintained extra-genomically in host cells but function in the same manner as endogenous chromosomes, has broad and immediate applicability in the field of recombinant DNA. Applicant is entitled to claims of a scope commensurate with the far-reaching development which has been provided to the public for immediate and valuable use through the guidance of the instant specification.

Rebuttal of the specific issues raised in the office action.

Methods of producing transgenic animals of species other than murine species

It is alleged in the Office Action that the specification refers only to transgenic methodology pertaining to the production of transgenic mice and that the prior Office Action (Paper No. 9) cited references which support that transgene behavior in mice cannot be extrapolated to other species of animal. It is concluded that evidence providing a nexus between a transgenic mouse and other transgenic animals by use of satellite DNA-based artificial chromosomes is necessary to enable the breadth of the claimed methods.

As set forth in the following discussion, Applicant respectfully disagrees with these grounds for rejection of the claimed subject matter under 35 U.S.C. §112, first paragraph.

The specification teaches that the artificial chromosomes disclosed in the application can be used in known procedures, including the generation of transgenic animals, previously carried out with standard gene delivery vehicles .

Not only does the specification describe in extensive detail the preparation, characterization, isolation and manipulation of artificial chromosomes, it further teaches that any methods known in the art pertaining to introduction of foreign genes carried in traditional, standard sources (such as

genes harbored in expression vectors) into cells and use thereof for any variety of purposes, *e.g.*, the generation of transgenic animals, may be applied in similar fashion to the introduction and use of the artificial chromosomes. The application is replete with descriptions of numerous uses of the artificial chromosomes and includes references to reported procedures for transfer and use of heterologous genes as *examples* of the types of methods in which the artificial chromosomes may be used. Among the exemplary procedures in which the artificial chromosomes may be employed are methods for generating transgenic animals such as those reported in the literature for producing transgenic mice.

The state of the art and level of skill in the art in 1996 was such that transgenic animals, other than mice, comprising the artificial chromosomes disclosed in the application could have been generated based on the teachings present in the specification combined with known methods for producing transgenic animals. Certainly, as listed above, there were numerous reports in scientific and patent literature by 1996 of transgenic animals of a variety of species, including, but not limited to, mice, cows, sheep, goats, pigs, rabbits and insects.

Patents are written to enable those of **skill in the art** to practice the invention. Those of skill in the art are familiar with and well-versed in the practice of standard techniques in the art. Therefore, a patent need not disclose what is well known in the art (W.L. Gore & Assoc. v. Gorlock, Inc., 721 F.2d 1540, 1556, 220 USPQ 303, 315). Thus, although methods for generating transgenic animals of a variety of species were known in 1996, there is no requirement that each and every method be included or referenced in the application in order for the specification to be considered enabling for the claimed methods. In fact, a patent application is considered to include all that is known to those of skill in the art; thus, the subject application effectively

includes all procedures known by 1996 for generating transgenic animals, be it murine or any other species of animal.

As such, the assertion in the Office Action that the specification "only refers to transgenic methodology pertaining to the production of transgenic mice" is irrelevant to a consideration as to whether the specification enables the claimed methods. The specification teaches the key elements in generating a transgenic animal containing the artificial chromosomes and also teaches that the artificial chromosomes can be used in known methods of generating transgenic animals of any species, citing as an *example* known methods of producing transgenic mice. Whether additional known protocols are referenced in the specification simply has no bearing on enablement of the claimed methods.

It is also noted that it is alleged in the Office Action that the references in the specification to methodology pertaining to the production of transgenic mice "does not include the use of SATACs or other MACs." In fact, the specification clearly describes the use of the artificial chromosomes in the production of transgenic mice. The specification may include reference to known standard methodologies used in making transgenic mice, but in the context of specific descriptions as to how such methodologies may be applied to the generation of transgenic animals containing the artificial chromosomes [see, *e.g.*, EXAMPLE 14 beginning on page 106 of the application and, in particular, page 110 of the application]. For example, on page 110 of the application, the generation of a transgenic mouse containing the anti-HIV ribozyme gene within a satellite DNA-based artificial chromosome is described:

> Fertilized mouse embryos are microinjected (as described above) with megachromosomes (1-10 pL containing 0-1 chromosomes/pL) isolated from fusion cell line G3D5$^+$ or H1D3$^+$ (described above). The megachromosomes are isolated as described herein. Megachromosomes isolated from either cell line carry the anti-HIV ribozyme (ribozyme D) gene as well as the hygromycin-resistance and β-galactosidase genes. The injected embryos are then developed into transgenic mice as described above.

Alternatively, the megachromosome-containing cell line G3D5$^+$ or H1D3$^+$ is fused with mouse embryonic stem cells [see, e.g., U.S. Patent No. 5,453,357, commercially available; see Manipulating the Mouse Embryo, A Laboratory Manual (1994) Hogan et al., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 253-289] following standard procedures see also, e.g., Guide to Techniques in Mouse Development in Methods in Enzymology Vol. 25, Wassarman and De Pamphilis, eds. (1993), pages 803-932]. (It is also possible to deliver isolated megachromosomes into embryonic stem cells using the Microcell procedure [such as that described above].) The stem cells are cultured in the presence of a fibroblast [e.g., STO fibroblasts that are resistant to hygromycin and puromycin]. Cells of the resultant fusion cell line, which contains megachromosomes carrying the transgenes [i.e., anti-HIV ribozyme, hygromycin-resistance and β-galactosidase genes], are then transplanted into mouse blastocysts, which are in turn implanted into a surrogate mother female mouse where development into a transgenic mouse will occur.

In the Declaration under 37 C.F.R. §1.132 of Perez (filed March 31, 2000), Applicant provided the Patent Office with detailed results of the process of generating transgenic mice containing a satellite DNA-based artificial chromosome using procedures relating to the artificial chromosomes described in the application and applying to them procedures known to those of skill in the art. It is acknowledged in the Office Action that the Perez Declaration is persuasive with respect to the issue of whether the specification enables methods of using satellite DNA-based artificial chromosomes to produce transgenic mice expressing heterologous genes at detectable levels. It is respectfully submitted that the Perez Declaration should not be limited to being persuasive of enablement of methods of producing transgenic mice alone. The data presented in the Perez Declaration represent much more and provide basis for farther reaching conclusions.

The data provided in the Perez Declaration submitted March 31, 2000, demonstrate that by using methods and materials described in the subject application and standard methods known in the art, viable transgenic animals are generated containing satellite DNA-based artificial chromosomes carrying heterologous genes which are maintained as intact autonomous, stably replicating, extra-genomic elements that are transmitted through the germline.

The artificial chromosomes thus survive transfer into cells used in the generation of transgenic animals and exist in the animal cells in a functional manner without integration into the host genome and without adverse effect on viability of the animal. The specification, consistent with the results presented in the Perez Declaration, thus discloses and teaches a highly desirable gene delivery system with wide-ranging applicability. In fact, the artificial chromosomes, demonstrated to be functional in cells and germline transmissible, have a broader range of uses than traditional gene delivery vehicles because they do not integrate into the host genome, and thus avoid potentially harmful disruptions of the genomic structure, and they have an enormous capacity to carry multiple genes and large genes into cells. Nothing in the data presented in the Perez Declaration or the specification provides any indication that the artificial chromosomes could not be used in the generation of other transgenic animals. On the contrary, all evidence presented by the Applicant strongly supports wide-ranging use of the artificial chromosomes.

Although the Patent Office has failed to provide relevant evidence that the breadth of the claimed methods is not enabled by the specification, data presented in the application and by way of Declaration provides ample, actual evidence that the teachings of the specification are commensurate with the scope of the claimed methods.

When rejecting a claim under the enablement requirement of 35 U.S.C. §112, "the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by [the] claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement." In re Wright, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993).

It appears that the reasoning on which the rejection of the claimed methods as not enabled by the specification is primarily based is an assertion that transgene behavior in mice cannot be extrapolated to other species of animals. In support of this assertion [as set forth in the prior Office Action (Paper No. 9), pages 13-14], reference is made to (1) Wall [(1996) *Theriogenology 45*:57-68] which allegedly discloses the unpredictability of transgene behavior due to factors such as position effect and unidentified control elements (2) Kappel *et al.* (JE) which allegedly discloses the existence of cellular mechanisms that may alter the pattern of gene expression resulting from differential CpG methylation and (3) Strojek and Wagner (NF) which allegedly points out the unpredictability of expression in different species due to differential interaction of cis acting elements with trans-acting factors between species. It is respectfully submitted that the reasoning (and alleged supporting evidence) offered on pages 13 and 14 of the previous Office Action (Paper No. 9) in connection with the grounds asserted for rejecting the instant claims under 35 U.S.C. §112, first paragraph, fails to meet the Patent Office's burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by the claims is not adequately enabled by the description provided in the specification.

As noted in Applicant's response to the previous Office Action (Paper No. 10), the publications cited in the Office Action appear to relate to possible differences in the expression of **specific** transgenes of **specific sequence** in different species. However, the rejected claims are **NOT** directed to a method of expressing a particular heterologous gene sequence across species. Instead, the claimed methods are directed to production of a transgenic animal employing an artificial chromosome which can be used as a vehicle for introduction of any transgene into the host animal. The ability of any particular transgene to be expressed at certain levels in a variety of animal species is irrelevant to the issue of enablement of the rejected claims.

Furthermore, as pointed out in the response (Paper No. 10) to the previous Office Action, it is of significant interest to note that the passage in the Wall publication to which the Examiner refers states that aberrant expression patterns in some transgenic animals results from random integration of transgenes into the host genome (i.e., the position effect). Thus, for example, if a transgene "lands near highly active genes, the transgene's behavior may be influenced by endogenous genes." The position effect is one of the main barriers to successful heterologous gene expression that is overcome by the use of the artificial chromosomes in the claimed methods of transgenic animal production. Because the artificial chromosomes are maintained as independent, extra-genomic elements in host cells, they eliminate the unpredictability associated with standard methods of introducing foreign genes into host cells via uncontrolled, random integration into the host genome. Therefore, it appears that the Wall publication is actually supportive of the wide applicability of the artificial chromosomes in the generation of transgenic animals.

In addition, at odds with the assertion in the Office Action that transgene expression in mice cannot be extrapolated to other species of animals, there is evidence in the literature that "mice are routinely used to evaluate the gene constructs to be transferred into large animals" (see abstract of Houdebine (1994) *J. Biotechnol. 34*:269-287, a copy of which is provided herewith). While analysis of hybrid genes in transgenic mice may not always reflect the expression levels in livestock animals [see, *e.g.*, Drohan (1992) *J. Cell. Biochem. 49*:111-112, page 111, right column (copy provided herewith)], it appears that mice are nonetheless sufficient to be used as a system to "audition" transgene expression prior to attempting transgenesis in other animals such as cattle, pigs and sheep. Except for transgene integration efficiency, several transgenic animal production efficiency parameters are similar for mice and farm animals (i.e., cattle, sheep and pigs) [see, *e.g.*, Wall *et al.*

(1992) *J. Cell. Biochem. 49*:113-120; page 116, right column and Table II on page 117; copy provided herewith].

PCT Application Publication No. WO93/25567 (copied provided herewith) is directed to production of recombinant polypeptides by bovine species and transgenic bovine methods. It is noted that in several of the Examples (*e.g.*, Example 21, page 106 and Example 23, page 112) relating to the disclosed methods of heterologous protein production in the milk of transgenic animals, particularly bovine, the system is first tested in transgenic mice.

The general strategies for producing transgenic livestock and mice are similar (see Wall *et al.* (1992) *J. Cell. Biochem. 49*:113-120, abstract). In PCT Application Publication No. WO93/25567, methods used in the development of transgenic bovine are described with reference to procedures for generating transgenic mice (see, *e.g.*, page 69, lines 4-9). The methods for producing transgenic livestock differ only in scale and minor detail from those used to produce transgenic mice. For example, the equipment needed for microinjection of livestock eggs is, for the most part, identical with that used for the mouse (see Wall *et al.* (1992) *J. Cell. Biochem. 49*:113-120, page 115, right column). Thus, although transgene expression in a transgenic mouse system may not necessarily be highly predictive of transgene expression in other animals, it appears that the those of skill in the art recognize sufficient similarities in transgenic mouse systems and other transgenic animal systems to (1) employ the same procedures used in making transgenic mice in the generation of other transgenic animals, *e.g.*, transgenic cattle, and (2) utilize transgenic mice as models in evaluating whether to apply the mouse-tested system to the development of other transgenic animals, particularly cattle.

A further Declaration of Perez demonstrates that using methods described in the application and standard methods of bovine embryo manipulation, it was possible to generate transgenic bovine embryos employing artificial chromosomes as disclosed in the application.

It is asserted in the Office Action that evidence providing a nexus between a transgenic mouse and other transgenic animals by use of satellite DNA-based artificial chromosomes is necessary to enable the breadth of the claimed methods. The further DECLARATION of Perez provided herewith describes the generation of transgenic bovine embryos using methods and materials disclosed in the subject application and standard methods as described in the DECLARATION. Transgenic bovine embryos were produced by microinjection of murine satellite DNA-based artificial chromosomes containing multiple copies of the *lacZ* (β-galactosidase) and *hph* (hygromycin phosphotransferase) genes into the pronucleus of fertilized bovine oocytes. Fluorescence *in situ* hybridization (FISH) analysis of blastocysts using probes specific for the artificial chromosome revealed that 27% of the embryos obtained after pronuclear injection scored positive for the presence of the artificial chromosome and that, on average, 27% of the cells of the positive embryos contained the artificial chromosomes as discrete chromosomes. These data demonstrate that satellite DNA-based artificial chromosomes as described in the subject application can be used in standard methods employed in the generation of transgenic animals to yield viable bovine embryos containing the artificial chromosomes in their cells.

The previous Perez Declaration (submitted March 31, 2000) demonstrated that using materials and methods described in the subject application and standard methods of transgenic animal production, transgenic mice containing satellite DNA-based artificial chromosomes that are transmitted through the germline could be produced. The further DECLARATION of Perez, provided herewith, demonstrates that, similarly, bovine embryos containing essentially

the same satellite DNA-based artificial chromosomes can be produced. The bovine embryos containing the artificial chromosomes should be suitable for transfer into a female recipient cows for development into transgenic cows. Although certain of the procedures utilized in generation of the transgenic bovine embryos are somewhat specific for production of transgenic bovine (for example, *in vitro* fertilization of the bovine oocyte), many of the basic oocyte handling and microinjection procedures were similar or identical to those used in generation of transgenic mice. As discussed above, those of skill in the art of transgenic animal generation recognize similarities in the production of transgenic mice and other transgenic animals, particularly bovine species. Clearly, the results described in the DECLARATION of Perez submitted herewith are supportive of a correlation between mouse and other transgenic animal systems as it was possible to generate transgenic bovine embryos just as it was possible to generate transgenic mouse embryos as demonstrated in the previous Perez Declaration. Therefore, it is respectfully submitted that Applicant has provided ample evidence that, as set forth in the subject application, the artificial chromosomes taught therein may be used in methods of producing transgenic animals of a variety of species.

The introduction of artificial chromosomes into ES cells

It is alleged on page 5 of the Office Action claims specifying an artificial chromosome contained within an embryonic stem cell should be limited to mouse embryonic stem cells since these are the only embryonic stem cells that have been shown to give rise to germline tissues and the whole animal. In the interests of advancing prosecution, claims have been amended thereby rendering any rejection based on this allegation moot.

Claims 42, 68-70 and 75-81

Claims 42, 68-70 and 75-81 are directed to transgenic animals comprising artificial chromosomes. The claims stand rejected under 35 U.S.C. §112, first paragraph. It is asserted in the Office Action that in light of the

specification, the claimed invention is properly interpreted with regard to the disclosed use of the claimed transgenic animals for conveying a state of disease resistance. It is concluded that because the specification as well as the evidence presented in the previous Perez Declaration allegedly fail to support that expression of any gene would result in immunoprotection, the specification fails to enable the claimed transgenic animals.

In the interests of advancing prosecution, the rejected claims directed to transgenic animals have been canceled thereby rendering any rejection based on this allegation moot. Applicant does not concede that the basis given in the Office Action for this rejection is proper, and the cancelled claims will be pursued in a continuing application.

THE REJECTION OF CLAIMS 32-44, 59 and 60 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 32-44, 59, 60 and 64-78 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Specifically, it is asserted in the Office Action that it is a huge leap to go from exposing a cell to conditions and the production of a transgenic animal, and thus more is required, including steps such as introducing an embryonic stem cell or fertilized ovum comprising a satellite DNA-based artificial chromosome into an embryo, transplanting the embryo into a recipient animal, allowing the embryo to develop to term and identifying a transgenic animal comprising the artificial chromosome.

The pending independent claims each include steps of introducing a cell comprising an artificial chromosome into a female animal and allowing the cell to develop into a transgenic animal comprising the artificial chromosome. It is respectfully submitted that the essential steps of the claimed methods are therefore included in the claims.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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